1961

The Relationship of Dopamine to Blood Pressure and Monoamine Oxidase Activity in Hypertensive Rats

David Rockwell De Fanti
University of Rhode Island

THE RELATIONSHIP OF DOPAMINE TO BLOOD PRESSURE AND MONOAMINE OXIDASE ACTIVITY IN HYPERTENSIVE RATS

BY

DAVID ROCKWELL DE FANTI

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

(Pharmacology)

UNIVERSITY OF RHODE ISLAND

1961

DOCTOR OF PHILOSOPHY THESIS

OF

DAVID ROCKWELL DE FANTI

Approved:

Thesis Committee:

Chairman John J. Reffo

Robert W. Harmon

Pierre R. Smith

Dean of the Graduate School E. W. Hartung

UNIVERSITY OF RHODE ISLAND

1961

ACKNOWLEDGMENTS

The author wishes to express his sincere gratitude to Dr. John J. DeFeo, not only for his guidance and assistance during the investigation and preparation of this manuscript, but also for his guidance and inspiration throughout the entire period of doctoral study.

The author wishes to express his sincere appreciation to Joel S. O'Connor for his assistance and advice concerning the statistical analysis contained herein.

The author wishes to express his gratitude to the United States Public Health Service for its support of this investigation through Research Fellowships MF-10,527 and MF-10,527-CL.

ABSTRACT

The relationship among urinary dopamine levels, arterial blood pressure and kidney monoamine oxidase activity was studied in hypertensive male rats.

The rats were rendered hypertensive by two operations; right nephrectomy followed in two weeks by contralateral renal arterial compression.

The blood pressures rose steadily throughout the first 11 weeks following the second operation and at the end of this period leveled off at approximately 180-185 mm Hg.

Dopamine was extracted from pooled urine samples by ion exchange techniques and measured by spectrofluorimetric methods.

Dopamine levels (mean from pooled group data) rose throughout the experimental period from a control value of 673 ± 166 µg/l to 1022 ± 449 µg/l. The rising pattern of dopamine was interrupted by sharp decreases during the eleventh and thirteenth weeks.

A linear correlation, excluding control values and values from the last week of the investigation, was conducted by regressing blood pressure on logarithm dopamine concentration. The analysis which was valid only for dopamine concentration ranging from 500-1000 µg/l resulted in a regression coefficient of 0.85 and a coefficient of determination of 0.72, indicating a fair degree of linear association between the two variables.

Monoamine oxidase activity dropped off sharply following the second operation, went through what appeared to be a compensatory rise, and then decreased to 40 percent of its original activity at the end of the investigational period.

In considering the interrelationship among the three variables, a definite correlation between dopamine and the blood pressure was shown to exist; however, a clear relationship between dopamine and monoamine oxidase inhibition was not established.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I. INTRODUCTION

The catecholamines, epinephrine, norepinephrine, and hydroxytyramine (dopamine) have often been implicated directly and indirectly with arterial hypertension.

It is known that they and some of their physiological metabolites are pressor to varying degrees and, therefore, capable of eliciting a rise in blood pressure. It is conceivable then that an excess of one or more of these chemicals occurring in the circulation could maintain, or at least support, a hypertensive condition.

The catecholamines, if they are a contributing factor to the hypertensive state, in all probability do so in a supporting role by helping to maintain the hypertensive condition rather than by actually being the original cause.

How could one of these catecholamines come to exist in concentrations in excess of normal physiological levels? Perhaps this could be brought about through disease interfering with one or more of the enzyme systems necessary for metabolizing the compounds.

This investigation is concerned with studying in particular one of these catecholamines, dopamine; one of the enzymes, monoamine oxidase; and the enzyme system, oxidative deamination responsible in part for its physiological disposition.

The problem is approached by studying the relationships between arterial blood pressure, urinary dopamine levels and kidney monoamine oxidase activity in rats with experimentally induced renal hypertension.

II. REVIEW OF LITERATURE

Arterial hypertension, whether as the result of a disease or produced by experimental methods, has become of extreme interest in the past twenty-five years. The role of the kidneys, the participation of nervous and endocrine systems, and the role of humoral pressor mechanisms in causing and maintaining hypertension have opened the road for many investigations.

More than one hundred years ago Richard Bright made the observation that hypertrophy of the heart, and by implication hypertension, often was the result of primary kidney disease in man (Goldring, 1946). This question in itself is greatly debated as hypertension is now known to exist without apparent primary renal disease. The mechanism of hypertension can best be studied by observing its early stages as they develop in induced experimental hypertension in animals.

The fact that hypertension was in some way associated with renal disease led early investigators to turn to the kidney. In 1879, Grawitz and Isreal, by partial nephrectomy of rabbits, demonstrated hypertrophy of the heart which they attributed to hypertension (Goldring, 1946). Passler and Heinke in 1905 repeated the procedure in dogs and confirmed Grawitz' and Isreal's work by actually demonstrating increased blood pressure. Alwens in 1909 compressed both kidneys by use of oncometers but reported equivocal results. Brudgman and Hirose reported negative results after acute partial occlusion of the renal arteries (Goldring, 1946). Until 1934 experimentally sustained hypertension had not been successfully produced.

Goldblatt et al. (1934) indicated that in dogs, at least, ischemia localized to the kidneys is a sufficient condition for the production of

persistently elevated systolic blood pressure. These investigators were the first to show that compression of the renal artery with a specially devised, adjustable, silver clamp, consistently produced a pronounced and permanent hypertension. The resulting ischemia of one kidney, with the other remaining normal, caused a moderate elevation of blood pressure which commenced three or four days after the operation. This persisted for only a short period of time, usually about a month. On the other hand, if one kidney was clamped and the other removed, or if both were clamped, permanent hypertension resulted. The investigators attempted several methods for producing an ischemic kidney, such as severe constriction of both renal arteries with short intervals between operations, almost complete constriction of both renal arteries with short intervals between operations, and moderate constriction of both renal arteries. Sustained hypertension was demonstrated in all of these with the exception of the latter, in which the hypertension was not great enough to be of experimental value. They also tested the blood pressure following ischemia of the femoral arteries following excision of the right suprarenal gland and degeneration of the left suprarenal gland. In the above three tests no significant change in blood pressure was observed.

In the same year Grollman et al. (1934) found that unilateral nephrectomy or the application of cloth or collodion to one kidney caused hypertension in a small percentage of normal rats. The subsequent removal of the kidney to which the cloth or collodion had been applied did not result in a significant decline of elevated blood pressure. Total nephrectomy in animals surviving long enough tended to cause an elevation of blood pressure. This is interpreted by Grollman and his coworkers as indicative that hypertension is due to an incipient deficiency

caused by removal of functional tissue rather than by release of a pressor substance.

Dury (1938) introduced a new method for causing hypertension by renal insufficiency. This was accomplished by placing a fine loop of silk thread around the left renal artery of the rabbit, then placing a piece of wire over the renal artery and tying the thread around the artery and the wire and then removing the wire. Thus, the patent diameter of the artery could be controlled. Page (1939a,b) was able to sustain arterial hypertension for some five months by wrapping first one kidney, then two weeks later the other, in cellophane soaked with alcohol. Grollman (1944) introduced a simple procedure for producing hypertension by exposing the kidney through the back and applying a ligature tautly around the three poles of the organ. The hypertension elicited by this method is not as severe and as persistent as that obtained by renal constriction; however, it does eliminate interference with the excretory process and is less apt to form a malignant type of hypertension.

It is difficult to study hypertensive methods without immediately becoming involved in the mechanism that causes acute and chronic hypertension. Tiegerstedt and Bergman (1898) obtained a pressor substance from normal kidney tissue of rats and postulated that in the case of defective kidney excretion there was an accumulation of this substance in the blood. Many believe that the initial cause of hypertension following constriction of the kidney is due to a release of a substance from the kidney, which while in itself is not pressor in action may be the precursor, or better still, the activator of a pressor substance. This is known as renin. A simple scheme for such a mechanism is shown in figure 1.

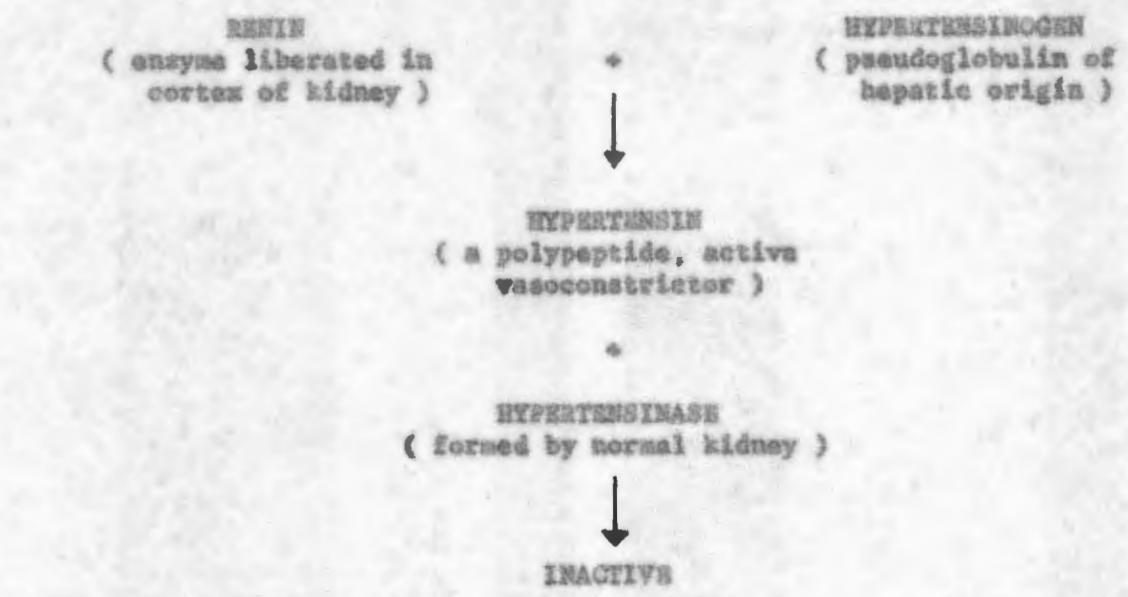

Fig. 1. Pathway for the liberation and destruction of renin (Best and Taylor, 1956)

According to Houdobro and Menendez (1942) renin is a protein enzyme produced in the kidney which interacts with a blood globulin (hypertensinogen) to form an active vasoconstrictor (hypertensin) which by its action on the peripheral vessels and on the heart causes a rise in blood pressure. Menendez (1956) states that the reaction is in all probability enzymatic and has an optimal pH of 7.5-8.5 and an optimal temperature of 37-39° C.

The investigations of Collins (1936) seemed to favor the theory of a release of a pressor principle. His studies eliminated the possibility that a nervous reflex originating from the kidneys affected the vasomotor apparatus, which thereby played a part in the origin of experimental hypertension, by demonstrating that hypertension was produced when the kidneys were completely denervated. Houssay and Taquini (1938) demonstrated that hypertension is due to a humoral mechanism caused by a pressor substance in the blood known as angiotonin or hypertensin. This hypertensin proved to be the result of a chemical reaction between a pseudoglobulin of the plasma and a certain substance known as renin to be found in the kidney. Later, it was proved by Page et al. (1941) that renin has the properties of a proteolytic enzyme. Sapierstein et al. (1941) presented the concept that the blood of the normal animal with normal pulse pressure contains activator substance but no renin, whereas blood taken after hemorrhage when the pulse pressure may be low, contains demonstrable amounts of renin-like substance.

Not all investigators have been in accord as to what substance, if any, is released into the blood during hypertension. Grollman and Rule (1938) observing that hypertension persisted even after bilateral nephrectomy, postulated that hypertension was due to removal of functional tissue

rather than to a release of pressor substance. Grollman (1942), however, found that hypertension in one of a pair of parabiotic rats caused hypertension in the other member, in some instances, thus indicating the possibility of a flow of some pressor principle in the blood. He further observed in the rabbit as he had in the rat, that the available data supported the view that chronic hypertension resulted from a deficiency induced by removal of normal renal tissues and not from the formation of a pressor substance, although the latter may play a role in the acute experiment. Dock (1940) in work with pithed rabbits indicated that renin, like epinephrine caused a rise in arterial blood pressure and thus differed from the humoral agent causing chronic renal hypertension. Shipley et al. (1948) observed that the pressor principle was not demonstrated in the plasma of bilaterally nephrectomized cats and normal cats, and concluded that a moderately prolonged period of hypotension with diminished blood force and pressure in the kidneys was necessary for production of a pressor substance. The pressor principle caused a rise in blood pressure in nephrectomized cats but not in normal cats. They stated that the pressor principle appears to be distinct from renin, pepsitensin, angiotonin, hydroxytyramine, or tyramine because of the difference in contour of the pressor response, the duration of the pressor response, and the different conditions under which the pressor response is observed.

The endocrine glands have often been implicated in the production of hypertension. Page and Sweet (1937) postulated a partial endocrine influence as being involved in renal hypertension. In that year, Page and Sweet noticed that blood pressure dropped considerably in hypertensive dogs which had been hypophysectomized following renal ischemia. The drop was not nearly as great in normal non-hypertensive hypophysectomized dogs.

They believed the effect of hypophysectomy on hypertensive dogs an indirect one, and it was postulated that the response of the blood vessels to chemical stimuli from the kidneys with constricted renal arteries was reduced. This was attributed to the lack of secretions of the adrenal and thyroid glands.

Page (1939b) found that unilateral adrenalectomy caused a drop in pressure in hypertensive rats whereas removal of the other adrenal gland, a month later, caused an even greater drop in pressure.

Bessinger and Haberlin (1948) found that the condition of adrenal insufficiency did not appear to reduce the renin concentration of the ischemic kidney, but appeared to favor renin return to the contralateral non-constricted kidney. They further found that the absence of both adrenal glands in three dogs with unilateral renal ischemia and receiving maintainance doses of desoxycorticosterone acetate (DCA) did not alter or lower the renin content of the contralateral non-constricted kidney. The injection of DCA into nine dogs for three to six weeks following unilateral renal ischemia significantly decreased the extractable renin from the constricted kidney but did not change or alter the renin concentration of the contralateral kidney. The renin was significantly decreased in the kidneys of three normal dogs receiving DCA for five weeks.

Hall and Hall (1949) observed that blood pressure of rats rendered hypertensive by treatment with DCA continued to rise following total nephrectomy. Hypertension of this type is not dependent upon the adrenal mechanism for its maintainance and is not a consequence of renal vascular damage, which is a later development.

Mason et al. (1950) indicated a possible role of the adrenals in chronic hypertension. They found that DCA, one of the hormones of the

adrenal cortex, corrected for some of the deficiencies caused by adrenalectomy. It elicited functional and structural changes characteristic of hypertensive vascular disease. The blood pressure of unilateral nephrectomized rats given DCA rose some 50 mm Hg, higher than those not given the hormone.

Goldblatt (1951) found that after bilateral adrenalectomy no hypertension could be observed following ischemia of the kidney; however, if cortin was administered hypertension resulted. It seemed that hypertensinogen was reduced after the adrenalectomy. The cortical hormone seems to be needed for the production of the substrate upon which renin acts.

Brandt et al., (1951) found that salt hypertension in the rat persisted for at least four weeks after adrenalectomy. Nephrectomy did not abolish salt hypertension. These results were interpreted as meaning that hypertension developing from salt administration is dependent directly on a disturbance of fluid distribution and not mediated thru the kidney or adrenals, such as was indicated by Goldblatt (1951).

Sevy and Wakerlin (1953) state that the only endocrines which appear to be involved in renal hypertensive processes are the hypophysis and adrenal cortex. They state that renin, by stimulating the pituitary, indirectly produces a secretion of DCA from the adrenal cortex which in turn causes a decrease in renal renin concentration. This decrease is measured on the basis of absence of increased renin secretion. However, DCA administration does not seem to hinder hypertension, but on the other hand seems to enhance it.

Haynes et al. (1953) observed that the plasma level of hypertensinogen decreased after adrenalectomy and returned to normal with

administration of cortical extracts. Cortisone and Adrenocorticotropic Hormone (ACTH) in adequate doses may increase the blood pressure in patients with renal disease or in sensitized animals. An elevation in hypertensinogen has been found to occur in normal rats after ACTH, as well as estrogens, have been administered. The increase in hypertensinogen observed after administration of ACTH was presumably mediated by the adrenals, since it was not detected in experiments on adrenalectomized animals. An elevation in hypertensinogen was also seen after indirect stimulation by the injection of epinephrine and direct stimulation with cortisone, but not with DCA.

The above data indicate that both the anterior pituitary and adrenal cortex play some role in the hypertensive mechanism although their roles cannot be exactly determined from the varied results.

One can assume with reasonable probability that renin by its activation of hypertensin is partially responsible for hypertension in the acute experiment; however, the humoral theory of the cause and maintainance of chronic hypertension must, in the last analysis, rest upon the demonstration of the occurrence of vasopressor substances other than renin in the peripheral blood, as renin cannot be demonstrate in chronic hypertension. Renin was not found in the peripheral blood of normal hypertensive animals in amounts detectable by the methods employed by Page (1940). Page (1939) was unsuccessful in his search for alcohol-soluble vasopressor substances, particularly angiotonin, in the peripheral blood of hypertensive animals.

Perhaps, then, there are other substances pressor in nature which are responsible for the maintainance of hypertension. The precursors of epinephrine as well as epinephrine itself could conceivably be responsible for the occurrence of the hypertensive state as they are all known to

possess pressor properties. Should there be interference with the metabolism of these compounds an excess of one or more might occur in the peripheral circulation.

It is generally agreed that epinephrine is formed from tyrosine as shown in figure 2.

The first three compounds, phenylalanine, tyrosine, and 3,4-dihydroxyphenylalanine (DOPA) are in themselves physiologically inactive. The last three compounds, hydroxytyramine (dopamine), norepinephrine, and epinephrine represent the active catecholamines.

The first important step is the decarboxylation of DOPA to dopamine which is catalysed by l-dopa decarboxylase, the enzyme first described by Holtz et al. (1939). This was in agreement with the hypothesis for the stepwise formation of epinephrine formulated independently by Holtz (1939) and Blaschko (1939).

The first definite evidence in support of this was given by Denis et al., (1955,56) when they incubated radioactive ($\alpha^{14}C$) dopa with homogenates of bovine adrenal medullary extracts and isolated radioactive dopamine and norepinephrine. Further evidence for the sequence is the occurrence of l-dopa decarboxylase (Langemann, 1951) and of small amounts of dopamine (Goodall, 1951; Shepherd and West, 1953a) in the suprarenal medulla, and the demonstration that both in vivo and in vitro medullary tissue is able to convert ($\alpha^{14}C$)-l-tyrosine and ($\alpha^{14}C$)-l-dopa into the equally labeled amines dopamine, norepinephrine and epinephrine (Hagen and Welch, 1956; Goodall and Kirshner, 1957; Kirshner and Goodall, 1956; Looper and Udenfriend, 1956).

Prior to 1957, the quantitative measurement of catecholamines was limited almost entirely to bioassay procedures (Gaddum, 1959), colorimetric

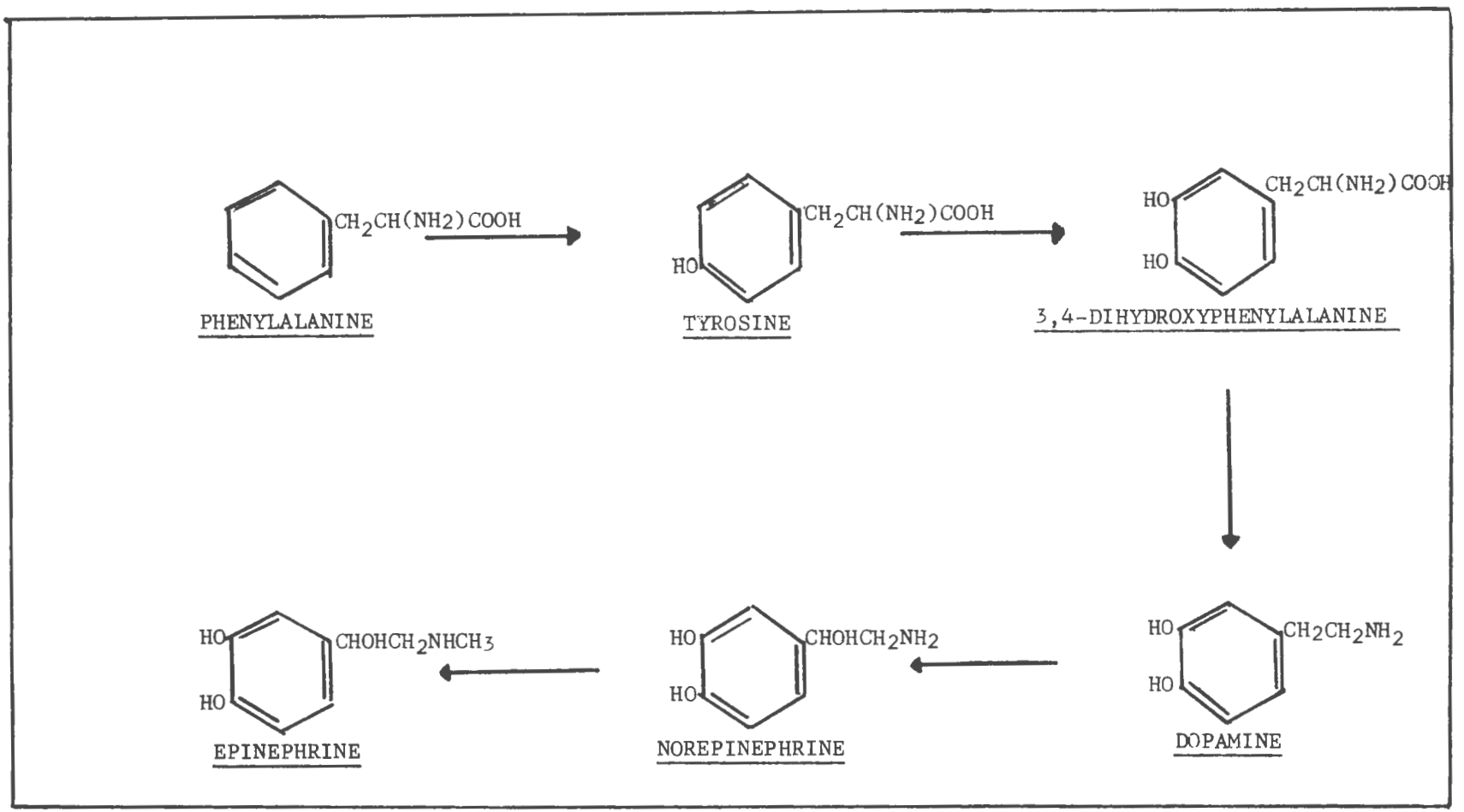

Fig. 2. Biosynthesis of the catecholamines.

techniques (Udenfriend, 1959), and paper chromatography (James, 1948; Goodal, 1959; Shepherd and West, 1953b; and Gregersman, 1951).

These techniques although very useful in qualitative identifications were not sensitive enough for good quantitative determinations.

During the past few years, with the introduction of fluorimetric analysis and the instrumentation progress in this area (Bowman and Udenfriend, 1955; Dugan et al., 1957; Udenfriend, 1959) the identification of the precursors and the pathway for the formation of epinephrine has been clarified. Physiological mechanisms for the inactivation of the catecholamines have been confirmed and new pathways have been found to exist. Until recently oxidative deamination and glucuronide or sulfate conjugation had been believed to be almost entirely responsible for the deactivation of the catecholamines.

Armstrong et al. (1957) demonstrated that a major metabolic product of norepinephrine in man is 3-methoxy-4-hydroxymandelic acid. Axelrod and co-workers have described the O-methylation of administered catecholamines as well as the normal occurrence of metanephrine (3-O-methylepinephrine) and normetanephrine (3-O-methylnorepinephrine) in urine and tissues. In addition, these workers also demonstrated the enzyme that catalyses the O-methylation, catechol-O-methyl transferase (COMT). To examine the importance of deamination in epinephrine and norepinephrine metabolism, the effects of iproniazid, a monoamine oxidase (MAO) inhibitor, were studied. Treatment with iproniazid following administration of epinephrine and norepinephrine resulted in a twofold increase in the metanephrine and normetanephrine excreted. In addition the amount of 3-methoxy-4-hydroxymandelic acid excreted was markedly reduced. These results seemed to indicate that MAO was primarily involved in the oxidative

deamination of normetanephrine and metanephrine and not in the deamination of the epinephrine and norepinephrine. In contrast to the above, treatment with iproniazid following administration of dopamine, resulted in fivefold increase in the O-methylated metabolite (methoxytyramine), thus indicating in the case of this particular catecholamine the primary route of metabolism is oxidative deamination (Axelrod et al., 1958a,b; Axelrod, 1957; Axelrod, 1959).

Goldstein et al. (1959) concluded that epinephrine and norepinephrine undergo O-methylation without deamination and were excreted mainly as metanephrine and normetanephrine and while dopamine undergoes methylation and deamination and was excreted mainly as 3-methoxy-4-hydroxyphenylacetic acid. These investigators found that when rats were treated with iproniazid, a MAO inhibitor, 31 percent of the administered radioactivity (3-hydroxyl α ^{14}C tyramine) was excreted as 3-methoxytyramine and 20 percent as 3-hydroxytyramine. This increase in the excretion of the intact amine after MAO inhibition demonstrates the involvment of the enzyme in the metabolism of dopamine. The excretion of the 3-methoxytyramine shows also that O-methylation does occur to the intact amine.

Sjoerdsma (1959) found that following administration of MAO inhibitors, the urinary excretion of epinephrine, norepinephrine, dopamine and possibly serotonin was not significantly increased. However, a marked increase in the urinary excretion of tryptamine, p-tyramine, and "p-tyramine-like" substances was observed. This indicated that still another efficient, alternate pathway for the physiological disposition of the catecholamines existed. Scheline (1960) indicated that the formation of p-tyramine substances was possible, by demonstrating that dehydroxylation of aromatic compounds does occur in the animal body.

Figures 3 and 4 depict the alternate pathways, with major enzymes involved, for the physiological inactivation of epinephrine, norepinephrine, and dopamine.

Information relating catecholamine levels to arterial hypertension has been sparse. Von Euler (1956) found that the norepinephrine levels were normal in 84 percent of 418 hypertensive patients tested. In the remaining 16 percent none exhibited what he called significant increases. Manger et al. (1959) demonstrated that circulating catecholamine levels were normal in hypertension. Weil-Malherbe and Bone (1957) reported an approximately twofold increase in epinephrine and norepinephrine and a fivefold increase in dopamine in hypertensive patients. The investigators also pointed out that the excretion of dopamine was extremely variable.

Schroeder and Adams (1941) demonstrated that the injection of a preparation of tyrosinase in suitable doses into rats and dogs exhibiting renal hypertension resulted in a reduction of blood pressure. Furthermore, they observed a difference in the response of normal animals, their blood pressure being less or not at all affected. The authors state that although the possibility exists that the hypotensive action of this enzyme is a non-specific one, the most probable explanation of the phenomenon is that some substance present in the hypertensive state is altered. Because tyrosinase acts only on compounds containing a mono- or di-hydroxybenzene structure in the molecule, such as the catecholamines, these substances are again implicated in the hypertensive process.

The kidney still receives extensive support as the producer of pressor materials in hypertension.

Bing (1941) reported that the production of a pressor substance presumably dopamine by decarboxylation of DOPA occurred in extracts of

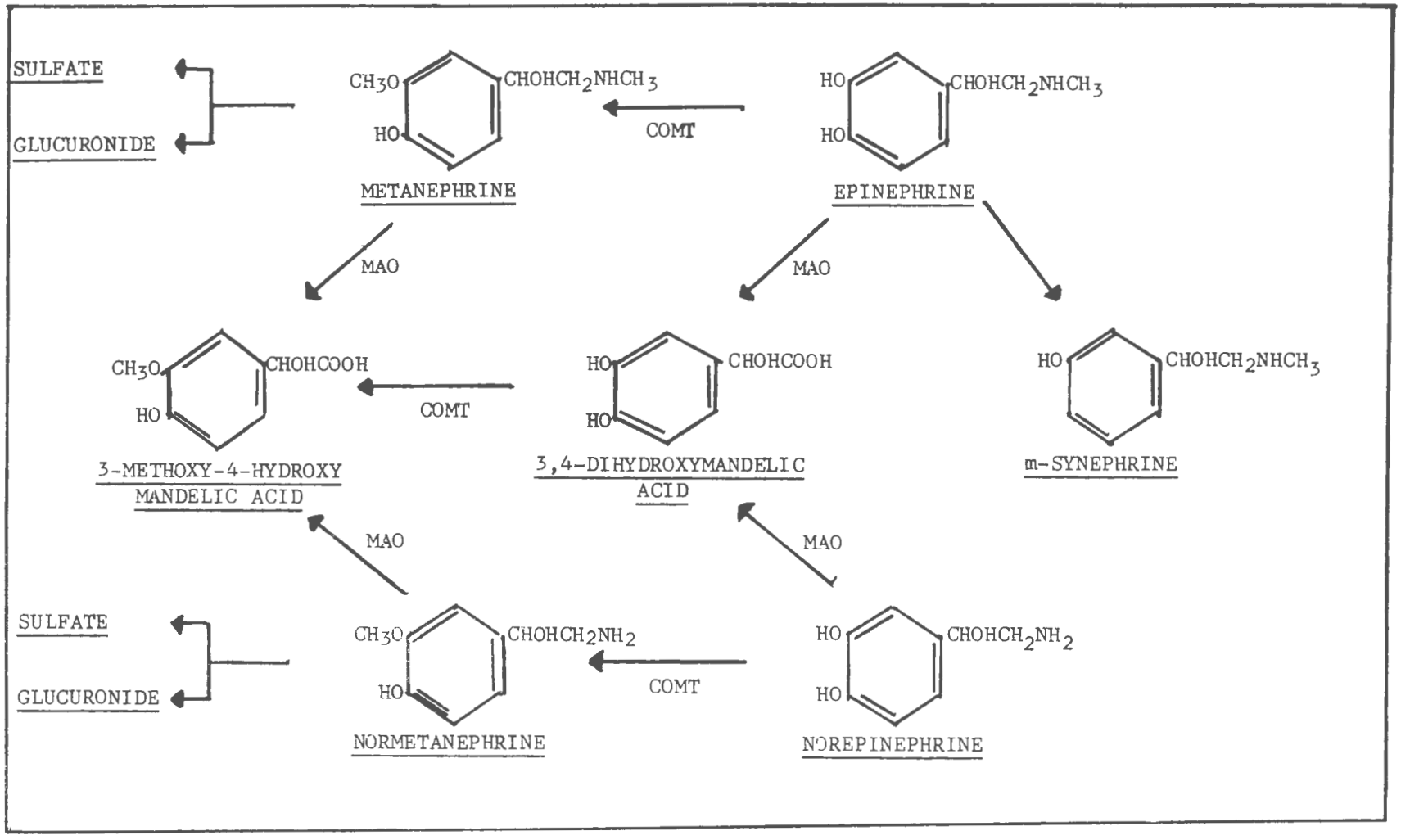

Fig. 3. Possible pathways for the metabolism of epinephrine and norepinephrine.

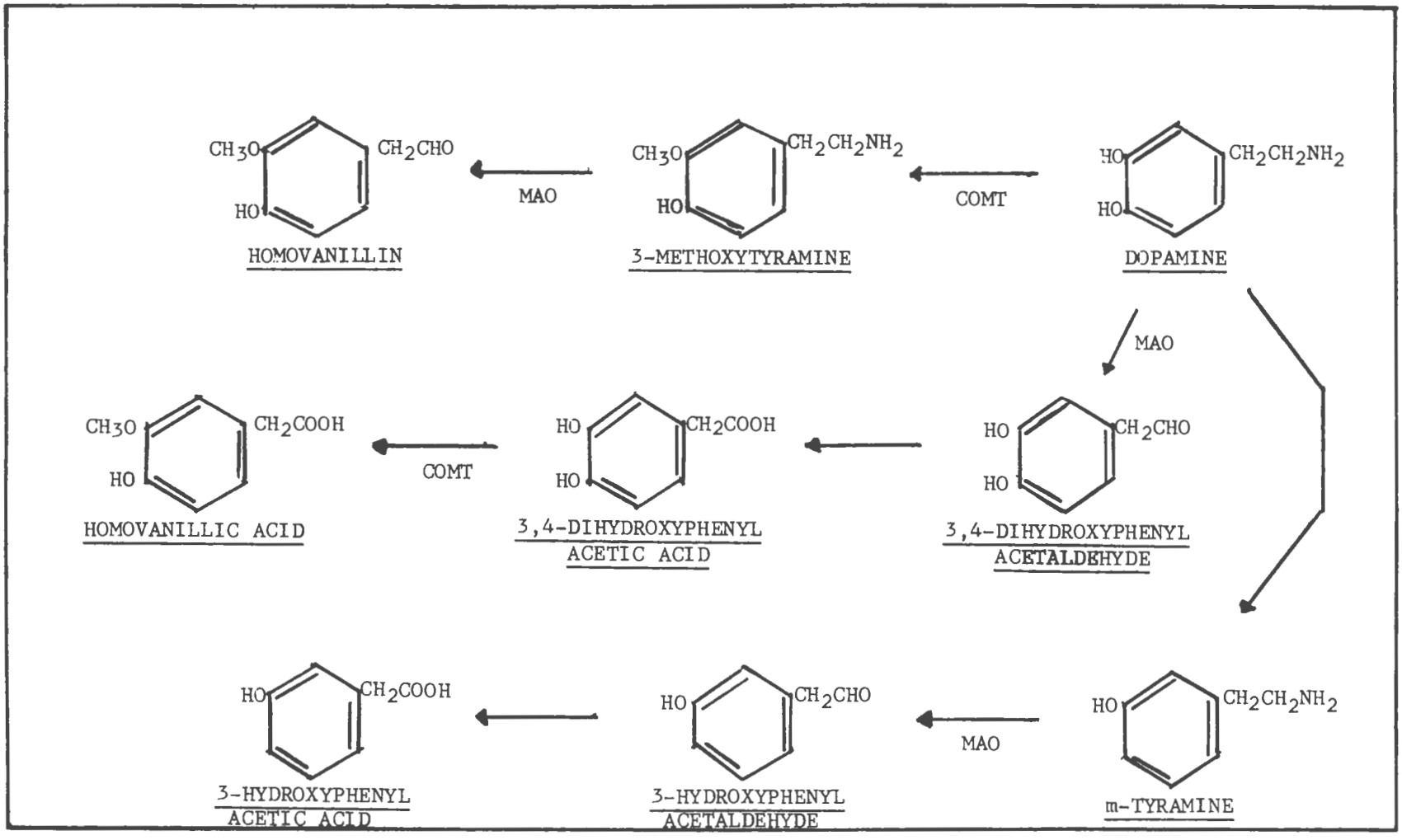

Fig. 4. Possible pathways for the metabolism of dopamine.

guinea pig kidneys incubated under anaerobic conditions. He noted that a similar reaction took place in the ischemic cat's kidney perfused with blood containing DOPA. As a control Ring also perfused liver and gut of the cat under analagous conditions and found no pressor properties. Schroeder (1942) presented evidence that deamination but not decarboxylation of certain amino acids is incomplete in kidneys deficient in their supply of oxygen. This was further substantiated by Giordano et al. (1959) who found a decrease in MAO activity in the kidneys of rabbits which were rendered hypertensive by renal ischemia. Since decarboxylation of amino acids leads in many instances to the formation of pressor amines, and oxidative deamination is necessary for the inactivation of these amines, it was believed that this process might be responsible for some varieties of arterial hypertension.

Blaschko (1952) demonstrated that MAO oxidizes dopamine at a rapid rate as it lacks the β-hydroxyl group possessed by the other catecholamines. Hagen and Welch (1956) indicated that MAO preferentially attacked dopamine. Dopamine appears then to have a tremendous affinity for MAO and therefore might be the primary physiological substrate for the enzyme (Blaschko, 1952).

In the case of renal insufficiency, the presence of continuing decarboxylation without deamination could lead to a buildup of dopamine which could not be metabolized.

Possibly conflicting with this theory is the report by Axelrod and Tomchick (1958) which stated that catechol-O-methyl transferase activity in the rat is the greatest in the liver and kidney. It was shown by Axelrod et al., (1958a,b) by the presence in rat urine of small amounts of methoxytyramine following administration of dopamine, that COMT

will metabolize dopamine to some extent.

Whether or not the principle route of metabolism of dopamine is O-methylation or oxidative deamination is still uncertain.

A controversial issue at the present time is which enzyme, COMT or MAO, is more important in the metabolism of the catecholamines. Does one control the storage and release of the amines while the other is mainly responsible for destruction of the circulating amines?

Spector et al. (1960a) proposed that monoamine oxidase was mainly responsible for the metabolism of norepinephrine in tissues where it may regulate the amounts of stored dopamine, norepinephrine, and 5-hydroxytryptamine.

Crout et al. (1960) proposed the hypothesis that MAO acts on tissue catecholamines and COMT on circulating catecholamines.

Spector et al. (1960b) in summary from further investigations postulated that MAO and COMT have different physiological roles. MAO is responsible for the metabolism of norepinephrine and 5-hydroxytryptamine in tissues, where it may regulate the level of stored amines; COMT is responsible for inactivation of catecholamines after their release into circulation.

The uncertainty concerning the two enzymes, MAO and COMT lies in the questions involving their distinct physiological functions. The fact that they are both extremely important in the disposition of the catecholamines is certain.

One question which has arisen recently is whether or not dopamine is pressor.

Hornykiewicz (1958) found that dopamine lowered blood pressure of the guinea pig. Administration of iproniazid, a MAO inhibitor, enhanced

this hypotensive effect. Burn and Rand (1958) found that dopamine causes a fall in the blood pressure of the guinea pig and cat under urethane anesthesia. When the guinea pig and cat are first injected with reserpine, which depletes the norepinephrine from vessel walls (Burn and Rand,1957) dopamine then becomes pressor. Upon the intravenous infusion of norepinephrine the depressor action of dopamine returned. The hypotensive action of dopamine seems to be quantitative in nature. It does not appear to be a question of whether or not dopamine is pressor or depressor but rather of competitive antagonism. A given amount of dopamine competes with norepinephrine for receptor sites, just enough to interfere with its action but not combining enough itself to elicit a pressor response (Burn and Rand, 1958).

Horowitz et al. (1960) demonstrated that the intravenous injection of dopamine into man elicited a rise in arterial blood pressure.

Progund et al. (1961) showed dopamine to be pressor in the rat. After an injection of DOPA an increase in blood pressure was observed. This was explained on the basis of increased dopamine production by decarboxylation of the DOPA. Following the administration of a dopa decarboxylase inhibitor the pressor response was lost.

Present evidence seems to indicate that dopamine is a pressor agent and that a major route of metabolism for this catecholamine is by oxidative deamination.

III. INVESTIGATION

A. OBJECTIVES

The factor or factors responsible for the cause and/or maintenance of chronic arterial hypertension, with the exception of structural changes in the blood vessels, have never been clearly demonstrated.

This investigation was undertaken in an attempt to elucidate one or more of these factors.

The specific objectives are:

1. To determine if there is an increase in the production of the catecholamines, in particular dopamine, in the urine of male rats rendered hypertensive by renal ischemia.

2. To determine if the arterial blood pressure is significantly dependent upon the increase in dopamine production.

3. To determine the physiological mechanism whereby the increase in dopamine comes to exist.

B. MATERIALS AND METHODS

1. Production of Experimental Hypertension

Male albino rats of the Sprague Dawley strain weighing 90-100 g were used for the investigation. Experimental hypertension was effected by renal insufficiency produced in the rats by the method of Goldblatt et al. (1934) as modified by Dury (1938).

The rats were subjected to two separate operations. The first operation involved removal of one kidney; the second, compression of the renal artery leading to the contralateral organ. The left kidney which possesses a somewhat longer renal artery was left intact for the second operation. Ether was the anesthetic in all operations.

Removal of the right kidney was accomplished by making a dorso-lateral incision about an inch in length midway along the spinal column on the dorsal side. The kidney could be easily located retroperitoneal to the abdominal cavity, lateral to the incision. By manipulation with the fingers the kidney was forced up through the incision and was readily accessible for surgical removal. The renal artery and vein were then doubly ligated with nylon thread, and kidney removed and immediately frozen for later use in enzyme studies.

Extreme caution was exhibited when removing the kidney in order to leave the adrenal gland intact. The incision through the muscle was closed with nylon suture and the incision in the skin closed with stainless steel wound clamps.

Two weeks subsequent to the first operation the renal artery to the left kidney was compressed to approximately one-third its normal diameter as described below.

The left renal artery was located by means of a one to one and one-half inch ventro-lateral incision in the lower abdominal area. By starving the animals for 24 hours prior to this operation, a minimum amount of operative manipulation of the intestines and stomach is required to obtain a clear working field about the kidney. The renal artery was then carefully separated from the renal vein and a wire stylus approximately one-third the diameter of the renal artery was laid across the vessel. A nylon suture was then placed around the wire and the artery and tied snuggly. The stylus was then carefully removed leaving the renal artery compressed to approximately one-third its original diameter. The incisions were closed in the manner previously described for the first operation.

The blood pressure of these animals was checked weekly by means of the photoelectric tensometer.[1]

2. Collection of Urine

At the start of the investigation, rats were divided into groups of eight. The animals were kept in pairs in cages equipped with stainless steel funnels for urine collections. Urine collections were made weekly, samples from individual groups pooled, filtered, and frozen for future analysis. Urine was collected in amber colored bottles containing 1.0 ml of 2.0 N sulfuric acid.

3. Extraction of Catecholamines from Urine

One mg/ml of ascorbic acid and two volumes of distilled water were added to a maximum of 15 ml of urine. The samples were then adjusted to pH 6.5 with 2.0 N sodium hydroxide (Crawford and Law, 1958). The catecholamines were extracted by passing the urine sample (pH 6.5) through a strongly acidic cationic exchange resin, Dowex 50W-X8, 200-400 Mesh.[2] Elution was performed with 2.0 N hydrochloric acid.

It has been demonstrated by Bertler et al., (1958) that the H^+ form of the cation exchange resin Dowex 50W-X8 will bind not only the catecholamines, epinephrine, norepinephrine, and dopamine, but also DOPA. It is extremely advantageous not to have DOPA present in the eluate as it has fluorescent characteristics very similar to those of dopamine and will interfere with the determination of this catecholamine. It has been further demonstrated by Bertler et al. (1958) that DOPA will pass readily through the Na^+ form of the exchange resin. It was

---

1. Photoelectric Tensometer, Metro Industries, Long Island City, N.Y.

2. Dowex 50W-X8, 200-400 Mesh is a product of the Dow Chemical Co. and was obtained from the J.T. Baker Chemical Co., Phillipsburg, N.J.

then necessary to convert the resin to the Na^+ form before use, as the Dowex 50W-X8 is supplied only in the H^+ form.

A Dowex 50 column with the dimensions 20 mm^2 x 12 mm was used. Before it was taken into use the resin was washed several times with 5.0 N sodium hydroxide followed with distilled water until the effluent became neutral. Ten ml of 1.0 N sodium acetate-acetic acid buffer, pH 6.0, was then passed through the column followed by 5.0 ml of distilled water. The column was now ready for use.

A 20 ml aliquot of the extract was passed through the column followed by 10.0 ml of distilled water. Elution was performed with 8.0 ml of 2.0 N hydrochloric acid. The flow rate was maintained at approximately 0.25 ml/minute.

4. Determination of Dopamine

The determination of dopamine was carried out according to the method of Carlsson and Waldeck (1958), a modification of the method of Bertler et al. (1958).

The pH of the eluate was adjusted to approximately 6.5 with 5.0 N potassium carbonate. Two ml of the neutralized eluate, 0.5 ml of phosphate buffer (pH 6.5), distilled water to give a total volume of 3.95 ml and 0.05 ml of 0.02 N iodine solution (0.252 g of iodine and 5.0 g of potassium iodide in 100 ml of water) were added to a test tube. After 5 minutes 0.5 ml of alkaline sulfite solution (5.04 g of sodium sulfite · $7H_2O$ in 10 ml of water, diluted to 100 ml with 5.0 N sodium hydroxide) was added followed in another 5 minutes by 0.6 ml of 5.0 N acetic acid.

The samples were then irradiated for 10 minutes under a short

wave ultra-violet light.[3]

The fluorescence of the samples was read on an Aminco-Bowman Spectrophotofluorometer.[4] The activation and fluorescent wavelengths were 325 mμ and 380 mμ respectively, and were determined using a standard solution of dopamine at a concentration of 0.5 μg/ml. The activation and fluorescent peaks are shown in figure 5.

A standard curve for known dopamine concentrations was run with each set of samples. The equations for the lines were determined according to Ostle (1956).

$$\sum Y = na + b\sum X$$

$$\sum XY = a\sum X + b\sum X^2$$

$$\hat{Y} = a + bx$$

A linear relationship such as reported by Carlsson (1958) was found to exist between concentrations of dopamine up to 0.4 μg/ml and the fluorescent intensity (figure 6).

The values for the unknown dopamine samples were then extrapolated from the calculated line, $\hat{Y} = a + bx$. Values for dopamine are reported in μg/l.

Internal standards were run at various intervals throughout the experimentation period. This was accomplished by the addition of known amounts of dopamine to the urine before it was processed.

The recovery values for internal dopamine standards are reported in table 1. It was found that the average recovery rate of dopamine was 74.52 ± 10.99 percent. The percent recovery appears somewhat low; however, Gey and Pletscher (1961) in using similar methods

---

3. Chromato-Vue, Black Light Eastern Corp., N.Y., N.Y. Peak emission 254 mμ.

4. Aminco-Bowman Spectrophotofluorometer, American Instrument Co., Inc., Silver Springs, Maryland. Specifications for the test: Xenon Lamp, Slit Arrangement number 5, Photomultiplier Tube 1P 28. Fused quartz cells were used.

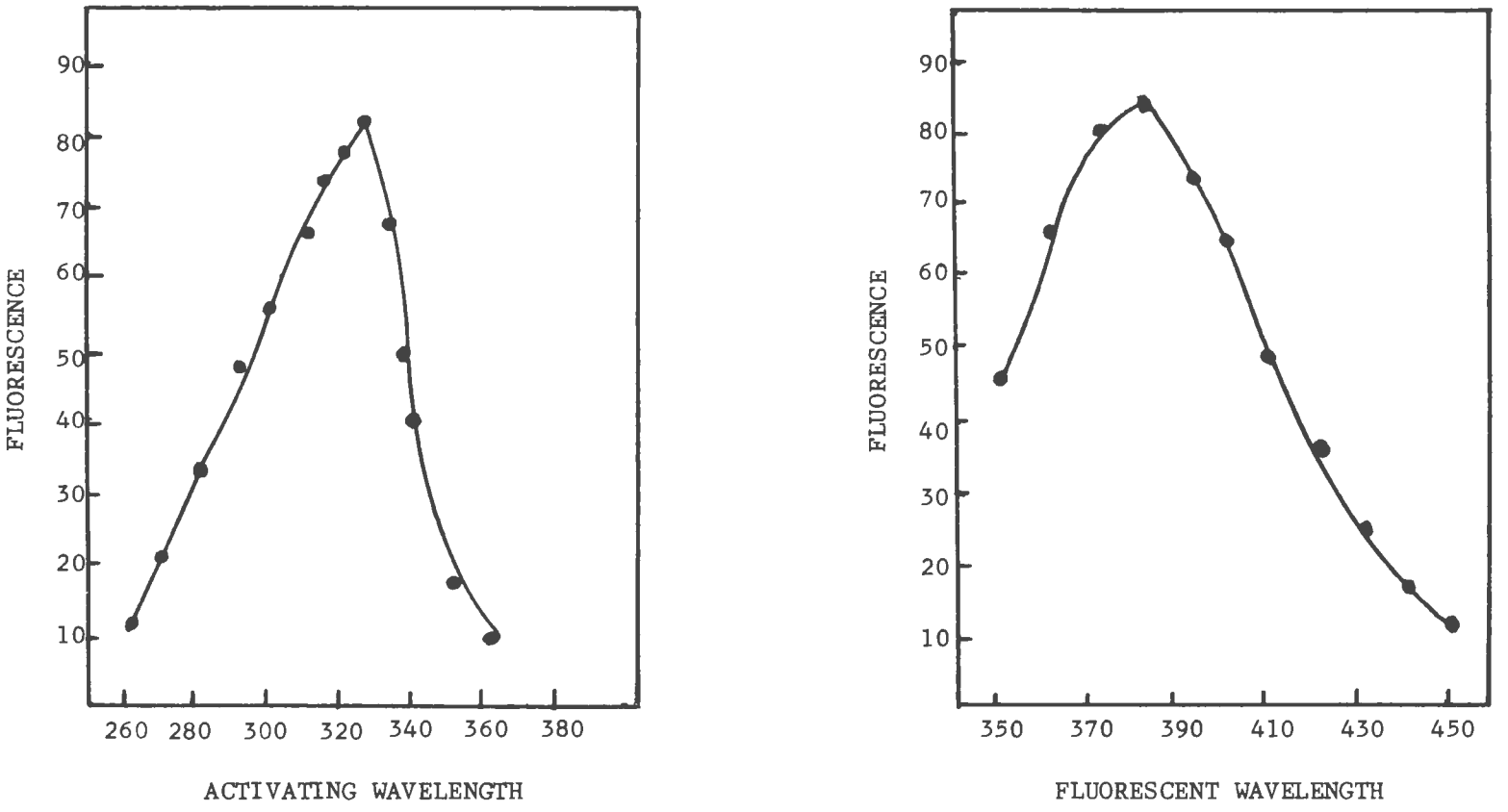

Fig. 5. Activation and fluorescence spectra of the fluorophore of dopamine.

Fluorescence is given in arbitrary units (meter multiplier value x % transmission x 100). When the activating wavelength was varied the fluorescent wavelength was set to 380 mμ. When the fluorescent wavelength was varied the activating wavelength was set to 325 mμ. The concentration of dopamine was 0.5 μg/ml.

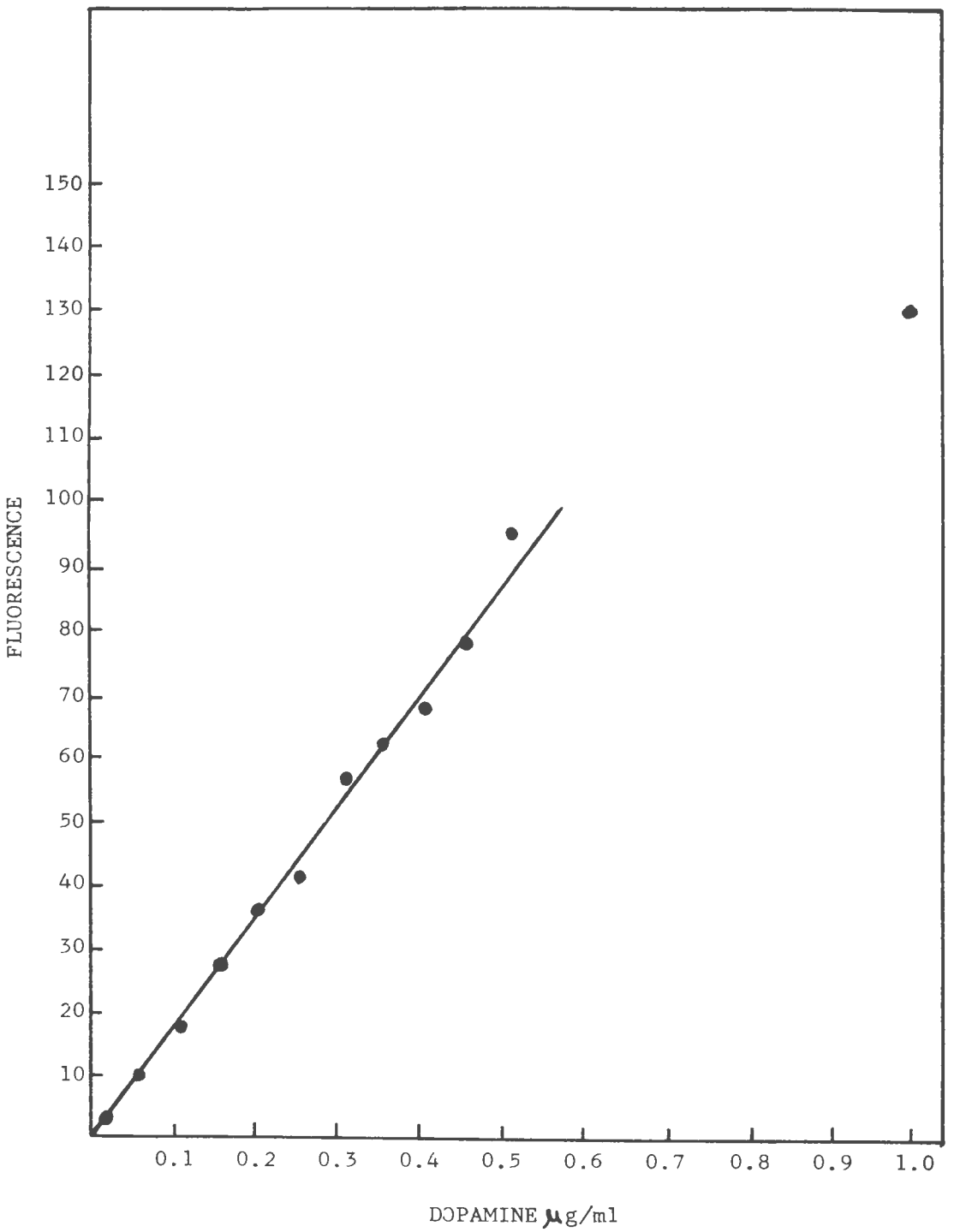

Fig. 6. Fluorescence intensity at varying concentrations of dopamine.

Fluorescence is given in arbitrary units (meter multiplier value x % transmission x 100). Activating wavelength 325 mµ; fluorescent wavelength 380 mµ.

TABLE 1

Percent recovery[a] of dopamine added to urine samples

Endogenous Dopamine μg/l	Dopamine Added μg/l	Total Dopamine Calculated μg/l	Total Dopamine Found μg/l	Percent Recovery
1147	1000	2147	2008	93.53
736	1000	1736	1382	79.61
840	400	1240	1006	81.13
845	2000	2845	1929	67.80
679	1000	1679	1014	60.39
599	1000	1599	1098	68.67
618	1000	1618	1141	70.52

[a] Average recovery = 74.52 %
Average recovery ± standard deviation = 74.52 ± 10.99 %

reported recovery of dopamine added to brain homogenates as low as 60 percent.

The preceeding method was chosen for the estimation of dopamine as it afforded a good procedure for differential analysis of this particular catecholamine even in the presence of the other catecholamines.

It was demonstrated by Bertler et al. (1958) that epinephrine and norepinephrine are quantitatively eluted by 1.0 N hydrochloric acid whereas dopamine is bound more strongly by the resin and can be eluted for the most part by 2.0 N hydrochloric acid.

Table 2 gives results from two columns eluted first with 8.0 ml of 1.0 N hydrochloric acid followed by 8.0 ml of 2.0 N hydrochloric acid.

TABLE 2

Percent dopamine eluted with 2.0 N hydrochloric acid.

Number	Dopamine µg/l		Percent Dopamine in Second Fraction
	1.0 N HCl	2.0 N HCl	
1	313	1069	77.35
2	391	740	65.45

Determinations of norepinephrine and epinephrine were attempted during the early stages of the investigation. The extraction procedure was the same as that described for dopamine. Epinephrine and norepinephrine determinations were made on the 1.0 N hydrochloric acid eluate, according to the method described by Bertler et al. (1958) for the differential estimation of catecholamines.

The presence of epinephrine and norepinephrine could not be detected. It is believed that the amounts present were below the

sensitivity limits of the test. This was substantiated by the fact that when 2.0μg/ml of both epinephrine and norepinephrine were added to the urine before processing, recoveries of 123 percent and 75 percent respectively were obtained.

3. Paper Chromatography

The differential, qualitative identification of catecholamines fromurine by paper chromatography was attempted.

The catecholamines were extracted from the urine by the method of von Euler and Hellner (1951).

The pooled urine sample was brought to pH 3.0 by the dropwise addition of 2.0 N sulfuric acid. Twenty percent aluminum sulfate, 1.0 ml/100 ml urine, was then added. Five tenths normal sodium hydroxide was then added with continuous stirring until a pH of 7.6 was recorded and the aluminum hydroxide formation was complete. The solution was then centrifuged. The precipitate was washed twice with distilled water and then was dissolved in 2.0 N sulfuric acid. The pH of the resulting solution was adjusted to 3.5 with 0.5 N sodium hydroxide. Four volumes of equal parts of ethanol and acetone were then added in order to precipitate the salts. The solution was then placed in the refrigerator for several hours.

When the salting process was completed the solution was filtered and the filtrate evaporated in vacuo at 50° C to a volume corresponding to 25-30 ml original urine/ml. The pH, if necessary, was then adjusted to 3.5. The extract was then ready for use and was stored in the refrigerator.

The extraction and separation of the catecholamines was attempted by the method of Shepherd and West (1953b) using descending paper chromatography. Samples of the extract along with control solutions of dopamine, norepinephrine and epinephrine were spotted in amounts varying from 0.05 µg to 1.0 µg on Whatman # 1 paper. The strips were allowed to equilibrate approximately 24 hours in the external phase of an acetic acid-butanol-water (1:4:5) solvent system. The time allowed for extraction against both external and internal phases varied from 12-20 hours.

The chromatograms were removed from the tank, allowed to dry completely and developed with a potassium ferricyanide solution (0.40%) in a 0.5 M phosphate buffer (pH 7.4) (Shore and Olin, 1958).

Upon developing, the catecholamines could be identified by the appearance of a pink colored spot. This eventually faded but gave way to a fairly intense fluorescence when viewed under short wave ultraviolet light. Complete separation was easily obtained with the control spots; however, complete, distinct separation of the three catecholamines could not be obtained from the urine extracts. A large area, pinkish in color and possessing fluorescent properties, would appear with an Rf value corresponding to the range of Rf values obtained from control spots of dopamine, norepinephrine and epinephrine. It is possible here that interfering substances in the urine extract hindered complete separation of the compounds.

6. Biological Assay

In preliminary studies bioassay techniques were employed for determining the presence of the catecholamines in the urine.

Pooled urine samples were processed in exactly the same manner as described in the preceding section on chromatography. Varying

concentrations of control solutions of epinephrine, norepinephrine and dopamine as well as varying amounts of urine extracts were injected intravenously into the female cat and the blood pressure changes recorded. Sodium pentobarbital was used as the anesthetic. The arterial blood pressure was taken by means of carotid cannulation and recorded on a smoked drum kymograph by means of a writing lever attached through a mercury manometer.

Increases in blood pressure were observed following all injections. The changes when using the urine extracts were slight, increasing from 5-10 percent, and transient.

Through the increase in blood pressure and the nature of the excursions, the presence of catecholamines in the urine extracts was indicated; however, a quantitative, differential estimation could not be made.

7. Monoamine Oxidase Determinations

One animal from each group was sacrificed every 2 weeks by cervical dislocation. Their remaining kidneys were immediately removed and frozen.

Kidney monoamine oxidase levels were measured as described by Cotzias and Dole (1951). Instead of measuring oxygen utilization during oxidative deamination of amine substrates, this method determines the amount of ammonia produced during the reaction, employing Conway Units.[5] The method is specific for primary amine substrates.

---

5. Conway Units complete with cover slips were obtained from the Arthur H. Thomas Co., Philadelphia, Pennsylvania.

A digestion time of 1 hour was allowed for the enzyme substrate reaction. Three hours were allowed for the transfer of ammonia produced by the reaction into the center well of the Conway Unit.

At the end of the 3 hours, titration of the ammonia trapped in a boric acid indicator in the central well of a Conway Unit, was carried out under fluorescent light by the delivery of 0.0100 N hydrochloric acid from a micro-syringe to an endpoint which matched that obtained by placing 1.0 ml of the boric acid indicator solution in the central well of a duplicate Conway Unit.

A unit of monoamine oxidase activity was defined as the quantity capable of catalysing the production of ammonia at the rate of 1.0 µM/hour under the conditions of the test. If V_s and V_b are the volumes in ml of hydrochloric acid required for titration of the sample and of the blank (tyramine omitted) respectively, and t is the time of digestion in hours, the number of units of enzyme contained in the sample U is given by $U = 20(V_s - V_b)/t$. In all tests a tyramine blank was run (tissue omitted) and this value was subtracted from V_s.

C. RESULTS

All results, including values from the control group, values from the individual test groups, pooled data from the test groups and statistical analyses are presented under this section.

1. Data for the Control Group are presented in TABLE 3.

TABLE 3

Control Group. Relationship of urinary dopamine levels (pooled samples) to arterial blood pressure (group mean) and kidney monoamine oxidase activity in male rats.[a]

Week Following 1st Oper.	Dopamine µg/l	Blood Pressure mm Hg	Monoamine Oxidase Activity U x 10^4	Number of Animals
Control	980	125.00	23	8
		First Operation		
1		130.00		6
2	845	120.83		6
3	599	122.50		6
4	679	122.50	31	6
5	647	133.75	38	5
6	480	118.75		4
8	441	94.00	14	4

[a] Right nephrectomy

2. Data for the Individual Test Groups 1-6 are presented in TABLES 4-9.

TABLE 4

Group 1. Relationship of urinary dopamine levels (pooled samples) to arterial blood pressure (group mean) and kidney monoamine oxidase activity in hypertensive[a,b] male rats.

Week Following 2nd Oper.	Dopamine µg/l	Blood Pressure mm Hg	Monoamine Oxidase Activity U x 10^4	Number of Animals
First Operation				
Control[c]		127.50	31	8
Control	523			8
Control	711	122.50		8
Second Operation				
1	596	147.00		5
2	398	145.00		5
3		161.00		5
4	377	164.00	21	5
5	1010	185.00		4
6	823	200.00		4
7	839	195.00		4
8	1150	180.00		3
9	1317	186.67		3
10	931	182.67		3
11	234			2
12	383	170.00		2
15	221	175.00		2
18	97	170.00	40	2
22	296	160.00	34	1

[a] Right nephrectomy
[b] Left renal arterial compression
[c] Control samples and readings taken at 1 week intervals

TABLE 5

Group 2. Relationship of urinary dopamine levels (pooled samples) to arterial blood pressure (group mean) and kidney monoamine oxidase activity in hypertensive[a,b] male rats.

Week Following 2nd Oper.	Dopamine µg/l	Blood Pressure mm Hg	Monoamine Oxidase Activity U x 10^4	Number of Animals
Control[c]	479	124.29		7
		First Operation		
Control		124.29	23	7
		Second Operation		
1	568			2
2		150.00		2
3	675			2
4		155.00		2
5	541	165.00		2
6	579	152.50		2
7	971			2
8	769	170.00		2
9		165.00		2
11	653	155.00	25	2
19	570	170.00	25	1

[a] Right nephrectomy
[b] Left renal arterial compression
[c] Control samples and readings taken at 1 week intervals

TABLE 6

Group 3. Relationship of urinary dopamine levels (pooled samples) to arterial blood pressure (group mean) and kidney monoamine oxidase activity in hypertensive[a],[b] male rats.

Week Following 2nd Oper.	Dopamine µg/l	Blood Pressure mm Hg	Monoamine Oxidase Activity B x 10^4	Number of Animals
Control[c]	697	121.88		8
		First Operation		
Control		118.13	50	8
		Second Operation		
1	639	145.00		4
2	604	155.00	26	4
3	583	160.00		3
4	632	157.67		3
5	419	160.00		3
6	854	191.67	16	3
8	389	205.00		2
9	494	197.50		2
10	629	195.00		2
11	1277	180.00		2
12	1369	182.50	25	2
13	1505	180.00		1
14	764	180.00	19	1

[a] Right nephrectomy
[b] Left renal arterial compression
[c] Control samples and readings taken at 1 week intervals

TABLE 7

Group 4. Relationship of urinary dopamine levels (pooled samples) to arterial blood pressure (group mean) and kidney monoamine oxidase activity in hypertensive[a,b] male rats.

Week Following 2nd Oper.	Dopamine µg/l	Blood Pressure mm Hg	Monoamine Oxidase Activity U x 10^4	Number of Animals
Control[c]	1358			
Control		120.00		8
	First Operation			
Control	757		58	6
Control	875			6
Control	739	127.50		6
	Second Operation			
1	430	145.00		2
2	502	160.00		2
3	480	155.00		2
4	859	167.50		2
5	380	165.00		2
7	454	187.50		2
8	527	170.00		2
9	765	200.00	30	2
10	1512	190.00		1
11	1278	180.00		1
12	840	180.00		1

[a] Right nephrectomy
[b] Left renal arterial compression
[c] Control samples and readings taken at 1 week intervals

TABLE 8

Group B. Relationship of urinary dopamine levels (pooled samples) to arterial blood pressure (group mean) and kidney monoamine oxidase activity in hypertensive[a,b] male rats.

Week Following 2nd Oper.	Dopamine µg/l	Blood Pressure mm Hg	Monoamine Oxidase Activity U x 10^{-3}	Number of Animals
Control[c]	946			8
Control		122.13		8
	First Operation			
Control	954		30	8
Control	737			7
Control	468	124.29		7
	Second Operation			
1	661	134.17		6
2	625	151.83		6
3	559	177.20	22	6
4	514	182.00		5
5	854	186.00	44	5
6	626	187.50		4
7	976	182.50		4
8	1068	193.75	34	4
9	563	195.00		3
10	754	193.33		3
11	687	188.33	14	3
12	1032	195.00		2
13	455	210.00	9	2
19	542		25	1

[a] Right nephrectomy
[b] Left renal arterial compression
[c] Control samples and readings taken at 1 week intervals

TABLE 9

Group 6. Relationship of urinary dopamine levels (pooled samples) to arterial blood pressure (group mean) and kidney monoamine oxidase activity in hypertensive[a,b] male rats.

Week Following 2nd Oper.	Dopamine µg/l	Blood Pressure mm Hg	Monoamine Oxidase Activity U x 10^4	Number of Animals
Control[c]	906	116.25		
First Operation				
Control	669		34	8
Control	455			8
Control	610	110.83		6
Second Operation				
1	559	131.60		6
2	681	147.50	19	6
3	626	165.00		5
4	474	169.00		5
5	360	177.00	16	5
6	1042	191.51		4
7	921	185.00		4
8	1128	181.75	25	4
9	1013	198.33		3
10	961	201.67	26	3
11	926	190.00		2
12	786	180.00	18	2
14	556		14	1

a Right nephrectomy
b Left renal arterial compression
c Control samples and readings taken at 1 week intervals

5. Pooled Data from all Individual Test Groups are presented in TABLES 10 and 11 and FIGURE 7.

TABLE 10

Master table. Relationship of urinary dopamine levels, arterial blood pressure and monoamine oxidase activity.[a]

Week Following 2nd Oper.	Dopamine γ/l		Blood Pressure mm Hg		Monoamine Oxidase Activity U x 10^4	
	γ/l ± SD	Range	mm Hg ± SD	Range	U ± SD	Range
Control	675 ± 166	(479-927)	122 ± 4.3	(114-125)	34 ± 9.2	(25-50)
1	542 ± 108	(396-661)	140 ± 7.3	(131-147)		
2	562 ± 112	(398-681)	148 ± 10.4	(130-159)	22 ± 2.9	(19-26)
3	585 ± 75	(480-675)	164 ± 8.4	(155-177)		
4	572 ± 179	(377-859)	166 ± 9.6	(158-182)	25 ± 16.2	(16-44)
5	627 ± 251	(380-1010)	173 ± 11.2	(160-185)		
6	785 ± 187	(579-1042)	185 ± 18.5	(155-200)		
7	752 ± 250	(454-976)	188 ± 5.4	(183-195)	31 ± 5.5	(25-34)
8	858 ± 328	(589-1150)	183 ± 15.8	(170-205)		
9	831 ± 338	(496-1317)	196 ± 5.5	(187-200)		
10	950 ± 338	(629-1512)	195 ± 6.9	(183-202)	19 ± 5.6	(14-25)
11	757 ± 343	(254-1278)	183 ± 17.0	(155-200)		
12	1022 ± 449	(383-1569)	184 ± 9.6	(170-195)	15 ± 5.6	(9-19)
13	684 ± 565	(221-1509)	183 ± 10.4	(175-195)		

[a] All values represent means with standard deviations of 6 groups of male rats (tables 2-8) at the time specified in column one.

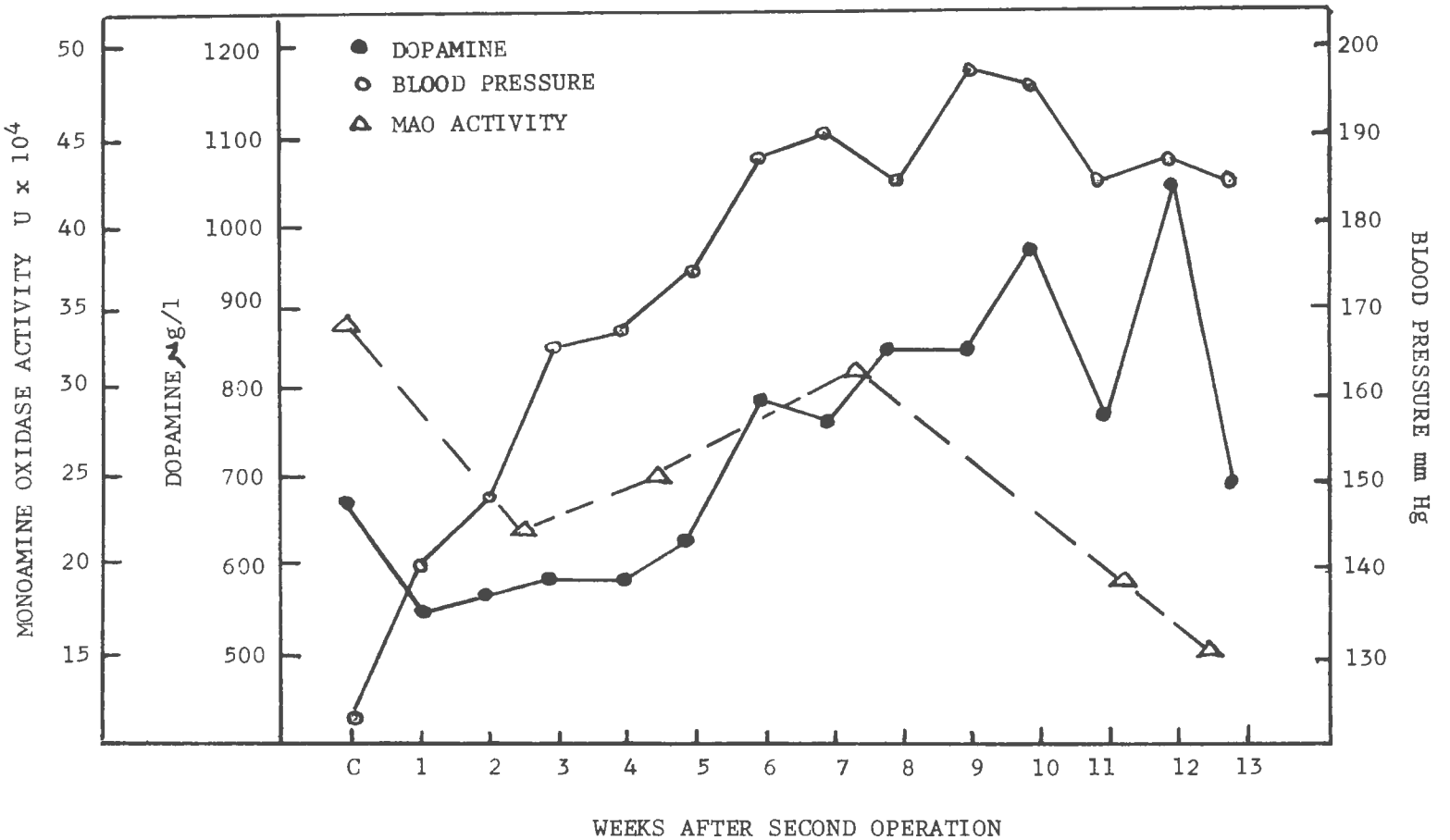

Fig. 7. Master graph. Relationships between urinary dopamine levels, arterial blood pressure, and monoamine oxidase activity.

All values represent means of six groups of male rats (table 10) at the times specified on the abscissa.

TABLE 11

Percent changes in urinary dopamine levels, arterial blood pressure, and monoamine oxidase activity.[a]

Week Following 2nd Oper.	Dopamine µg/l % Change	Blood Pressure mm Hg % Change	Monoamine Oxidase Activity U x 10^4 % Change
Control	0	0	0
1	-19.5	+14.8	
2	-16.5	+21.3	
			-35.5
3	-13.1	+34.4	
4	-15.1	+37.7	
			-26.5
5	- 6.8	+41.8	
6	+16.6	+51.7	
7	+11.7	+54.1	
			- 8.8
8	+24.5	+50.0	
9	+23.5	+60.7	
10	+41.2	+58.2	
			-44.1
11	+12.5	+50.0	
12	+51.9	+56.8	
			-55.9
13	+ 1.6	+50.0	

[a] All values represent means of six groups of male rats (table 10) at the time specified in column one.

4. A Regression Analysis, including a Test of the Hypothesis and Confidence Limits, of Blood Pressure on Logarithm Dopamine is presented in TABLE 12, FIGURE 8 and on PAGE 52.

TABLE 12

Data for the regression of blood pressure on logarithm dopamine[a,b]

Y Blood Pressure mm Hg	X Log. Dopamine Concentration
140.00	2.73400
148.00	2.74974
164.00	2.76716
166.00	2.75740
175.00	2.79727
185.00	2.89487
188.00	2.87622
183.00	2.93324
196.00	2.91908
193.00	2.97720
183.00	2.87910
184.00	3.00885

[a]This regression is valid for concentrations of dopamine ranging from 500-1000 µg/l and is independent of time.

[b]Blood pressure and dopamine values are taken from table 10. Control values and observations from the thirteenth week are omitted from this analysis.

Regression Analysis:

$a = -280.64$

$b = +159.57$

Regression equation: $Y = a + b \text{ Log } (X)$

$Y = -280.64 + b \text{ Log } (X)$

Regression coefficient (r): 0.8464

Coefficient of determination (r^2): 0.7201

Test of Hypothesis:[6]

H_o: $\rho = 0$ (Test of statistical dependence)

$t_{.05}(N-2) = 2.228$ (Percentage points of the t-distribution)

$|T| = 3.094$

Confidence Limits:[6]

$S_e^2 = 96.09$ (Standard error of the estimate)

$S_b = 51.65$ (Standard error of the regression coefficient of b)

$S_a = 90.48$ (Standard error of the regression coefficient of a)

$L_b = b \pm t_{.05}(N-2)S_b$

$L_1 = 230.09$
$L_2 = 89.03$

$L_a = a \pm t_{.05}(N-2)S_a$

$L_1 = 79.05$
$L_2 = -482.23$

6. All calculations concerned with the regression analysis, test of hypotheses, and confidence limits were performed according to Ostle (1954).

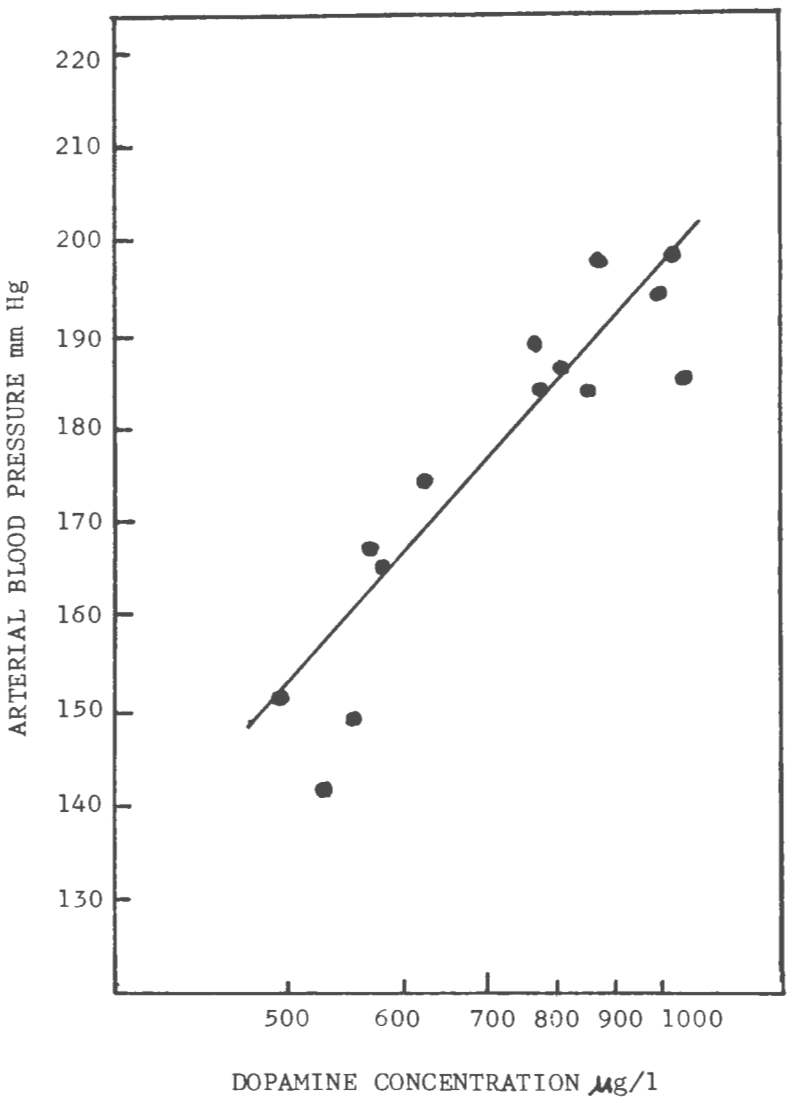

Fig. 8. Regression of arterial blood pressure on logarithm dopamine concentration.

Mean values of six groups of male rats. This regression holds for values of dopamine between 500 and 1000 µg/l.

IV. DISCUSSION

The method employed for inducing renal hypertension (Goldblatt et al., 1934; Dury, 1938) was found to be satisfactory.

During the first week subsequent to the second operation (fig. 7) the blood pressure began to rise sharply. During the ninth and tenth weeks the pressure reached its maximum (table 11), rising 60.7 and 58.2 percent respectively above control levels.

Following the tenth week the pressure dropped approximately 5 percent and leveled off. However, the values recorded subsequent to the tenth week were well within hypertensive limits.

The average control blood pressure in the test groups (table 10) was 122 $\pm$ 4.3 mm Hg and reached a maximum of 196 $\pm$ 5.3 mm Hg on the ninth week. In the control group (table 3) the average blood pressure before the first operation was 125 mm Hg and varied throughout the test period from 94 to 153.75 mm Hg.

The average urinary dopamine level in the normotensive animals was found to be 673 $\pm$ 166 μg/l (table 10). Throughout the experimental period, in the individual test groups, dopamine values ranged from a low of 97 μg/l (table 4) to a high of 1969 μg/l (table 6).

In the control group (table 3) dopamine values ranged from 980 μg/l at the start to 441 μg/l 8 weeks after the first operation. The initial value 980 μg/l was high as similarly observed in groups 4,5 & 6 (tables 7, 8 & 9) and could possibly be explained on the basis of a stress condition of the rats before becoming accustomed to their new environmental conditions. The blood pressures were not elevated at this point. Within groups 4, 5, & 6 (tables 7, 8, & 9) the control values dropped considerably following the first operation but prior to the second operation.

The standard deviations and ranges (table 10) were large and variable. This is not unusual as Weil-Malherbe and Bone (1957) found the excretion of dopamine in normotensive and hypertensive patients to be quite variable. Drujan et al. (1959) reported that dopamine excreted by man over a 24 hour period ranged from 26-395 µg.

The dopamine values (fig. 7) dropped sharply, approximately 20 percent (table 11), during the first week following the second operation. By the end of the first week the values had started to rise gradually and by the sixth week the rising trend had become definite. By the tenth week the dopamine levels had increased 41.2 percent (table 11). A sudden drop occurred during the eleventh week; however, a peak value 51.9 percent (table 11) above normal was reached on the twelfth week. The sudden decrease in dopamine concentrations on the thirteenth week cannot be explained fully on the basis of the available data; however, it should be noted (table 10) that the standard deviation (684 ± 565 µg/l) and the range (221-1505 µg/l) were very great at this particular time.

In individual test groups 3 & 4 (tables 6 & 7), the maximum dopamine levels were reached during the tenth to twelfth weeks. A sharp decrease occurred on the fourteenth and twelfth weeks respectively. In groups 3 & 4 a well defined rising pattern was noted from the eighth to thirteenth and seventh to eleventh weeks respectively.

In individual test groups 1 & 5 (tables 4 & 9), maximum dopamine levels were reached during the fifth to tenth weeks, somewhat sooner than was noted in the groups previously mentioned. Again, there was a sharp drop in dopamine levels on the tenth to eleventh weeks.

In group 2 (table 5) the dopamine concentration reached a maximum of 769 µg/l on the seventh week following the second operation; however,

the rise was not as prominent as that of the preceding groups. In this group the blood pressure reached 170 mm Hg which is also not as high as the maximum pressures noted in other groups.

In group 5 (table 8), the dopamine values reached maximum levels of 1068 and 1032 µg/l on the eighth and twelfth weeks respectively. The level fell quite sharply between the two peaks and again after the second peak.

Results from the regression analysis of blood pressure on logarithm of dopamine are shown in figure 8, table 12, and page 52. The analysis was conducted on the data pooled from all groups (table 10) excluding control values and observations from the thirteenth week. The analysis which was run independently of time is valid for values of dopamine ranging from approximately 500-1000 µg/l.

The regression coefficient indicated that there exists a degree of linear association between blood pressure and dopamine concentrations. The magnitude of this correlation was 85 percent.

The coefficient of determination indicated that 72 percent of the variations occurring in Y (Blood Pressure) could be explained on the basis of the linear regression of Y on X (Logarithm Dopamine Concentration).

In a test of the null hypothesis, $H_0: \rho = 0$, $T = 5.094$ was greater than $t_{.05}(N-2) = 2.228$, thus causing rejection of the hypothesis. The hypothesis, $H_0: \rho = 0$ states that the two variables present are statistically independent, thus rejection of the hypothesis in this case shows that Y (Blood Pressure) and X (Dopamine) are, in fact, statistically dependent. The percentage points of the t-distribution were taken at the 0.05 level and, therefore, the results of this particular test can be accepted with a 95 percent confidence.

Further tests concerning this analysis, show that, with 95 percent confidence, the true regression coefficient β lies between 89.05 and 230.09 and that the true regression coefficient κ lies between 79.05 and -482.25.

The results from the regression analysis indicate that there exists a fairly good degree of linear correlation between the variables (Blood Pressure and Dopamine) and that they are definitely dependent.

Evidence, contrary to the dependency of blood pressure on dopamine levels, is inferred from the fact that in certain groups previously mentioned control dopamine values were quite high; however, the blood pressure was not elevated. In these particular cases the dopamine levels were in all probability, not consistently elevated for any length of time.

Considering the entire six groups in general, it can be seen (fig. 7) (table 11) that the monoamine oxidase activity had dropped approximately 35 percent during the first 2-3 weeks following the second operation. During the third to fifth weeks, the activity had started to increase in what appears to be a compensatory reaction. By the fifth week the activity was decreased by 26.5 percent. The rise in activity continued until the seventh to eighth weeks and at this point was decreased by only 8.5 percent.

Subsequent to the eighth week, the monoamine oxidase activity fell rapidly and at the termination of the experiment had decreased to approximately 60 percent of the control value. The finding of the ultimate decrease in kidney monoamine oxidase activity following renal ischemia are in accord with the findings of Schroeder (1942) and Giordano et al. (1959).

The initial drop in monoamine oxidase activity, the subsequent rise, and the final decrease in activity were noted in individual test groups 1,

3, 5, & 6 (tables 4, 6, 8, & 9) as well as in the control group (table 3).

In group 2 (table 5) no significant changes in monoamine oxidase activity were noted. On the basis of the number of observations (2) in group 5 (table 7), the existence of an actual trend could not be seen.

From figure 7, it can be noted that during the second and eighth weeks there appears to exist a parallel between monoamine oxidase activity, dopamine, and blood pressure as all are increasing. After the eighth week the monoamine oxidase activity started to fall but the dopamine and blood pressure continued their rising trends.

The highest blood pressure values (table 11) were recorded during the ninth to twelfth weeks. During this period (table 11), the dopamine values were at their highest levels and the monoamine oxidase activity was falling off sharply.

The sudden drop of all three components during the final weeks of experimentation cannot be explained on the basis of available data.

Two possible explanations, in regard to the correlation between dopamine and monoamine oxidase activity, (the correlation between blood pressure and dopamine having been previously shown to exist), seemed to be suggested from the analysis of the data pooled from all six test groups.

The first is that the compensatory rise of monoamine oxidase activity is the result of increased dopamine levels. The dopamine levels having increased in the hypertensive state due to factors not elucidated from this investigation. This, as has been previously pointed out, can be seen (fig. 7) during the second to eighth week following the second operation. In group 6 (table 9) the dopamine values were at their highest values during the sixth to eleventh weeks; the blood pressure values were

also at their highest levels during this period. The monoamine oxidase activity was, however, undergoing a compensatory rise from the fifth to tenth weeks. In the control group (table 3) dopamine levels appeared to be completely independent of monoamine activity.

The second explanation is that dopamine levels increased by virtue of decreased monoamine oxidase activity. From figure 7 it can be noted that from the eighth to twelfth weeks dopamine values were still rising and during this period reached, along with the blood pressure values, their peak values. On the other hand, monoamine oxidase activity was dropping off sharply during this period. In support of this second explanation, it is shown in group 2 (table 5) that monoamine oxidase activity did not significantly change during the course of the experiment and that the dopamine levels rose only slightly. The blood pressure here reached a high of 170 mm Hg, lower than the maximum values obtained in other test groups. In group 5 (table 8) the dopamine and blood pressure values reached their highest levels during the fifth to thirteenth weeks. The monoamine oxidase activity was decreasing during this period.

Results from this investigation indicate that blood pressure in experimentally induced renal hypertension is dependent to a fair degree upon dopamine concentrations; however, the dopamine levels appear to be independent of monoamine oxidase activity. On the basis of this independence, the factors causing the dopamine increases cannot be explained from the investigation. The dopamine levels do, in most cases, reach their peak values after the monoamine oxidase activity has started to drop for the second and final time. This final decrease in monoamine oxidase activity could very possibly be eliciting an additive effect upon the dopamine levels.

Further investigation into the alternate enzyme systems capable of catalysing the metabolism of the catecholamines must be conducted before the increases in dopamine concentrations observed in this study can be fully explained.

V. SUMMARY AND CONCLUSIONS

The interrelationships of arterial blood pressure, urinary dopamine levels, and kidney monoamine oxidase activity in experimentally induced renal hypertension has been investigated.

Experimental hypertension was induced in male rats of the Wistar strain weighing 90-100 g. The blood pressure started to rise within the first week following the second operation and reached the "hypertensive level" at about the end of the third week. Pressures ranged on the average from 160-196 mm Hg and leveled off on about the eleventh week at approximately 180-189 mm Hg.

Bioassay and paper chromatographic techniques were at first used to determine catecholamine levels; however, these proved inadequate for quantitative, differential analysis.

Dopamine was extracted from pooled urine samples by ion exchange techniques and measured by spectrofluorimetric methods. The fluorimetric method used was found to be satisfactory with average recovery for dopamine added to urine samples being 74.52 $\pm$ 10.99 percent. The activating and fluorescent wavelengths for dopamine were found to be 325 mμ and 380 mμ respectively. A linear relationship between fluorescent intensity and dopamine concentrations from 0.05 μg/ml to 0.40 μg/ml was found to exist. This relationship quite often held for concentrations up to 1.0 μg/ml.

Dopamine levels rose throughout the experimental period from an average control value of 673 $\pm$ 166 μg/l to an average peak value of 1022 $\pm$ 449 μg/l. The excretion of dopamine was variable and the standard deviations and ranges were often large.

A simple correlation, excluding control values and observations from the thirteenth week, was run by regressing blood pressure on the

logarithm of dopamine. A regression coefficient of 0.85 indicated that there was a good degree of linear association between the two variants. The coefficient of determination, 0.72, showed that 72 percent of the variations occurring in blood pressure could be explained on the basis of the linear regression of blood pressure on dopamine. The regression was run independent of time and is valid for dopamine concentrations ranging from approximately 500-1000 µg/l.

Monoamine oxidase determinations were conducted on the kidneys removed during the first operation and on the kidneys from animals sacrificed at 2 week intervals during the experimentation period.

Monoamine oxidase activity dropped sharply following the second operation, went through what appeared to be a compensatory rise, and then decreased finally to approximately 40 percent of its original activity.

A definite relationship between blood pressure and dopamine was established. Dopamine values appeared to rise independently of monoamine oxidase activity, with exception of the final drop occurring after the compensatory rise. Here an additive effect of the decreasing monoamine oxidase activity on the increasing dopamine levels could be present. A clear relationship between monoamine oxidase inhibition and urinary dopamine levels was not established from this investigation.

VI. REFERENCES

Armstrong, M.D., McMillan, A. and Shaw, K.N.F.: 3-Methoxy-4-hydroxy-d-mandelic acid, a urinary metabolite of norepinephrine. Biochim. biophys. Acta 25:422, 1957.

Axelrod, J.: O-Methylation of epinephrine and other catechols in vivo and in vitro. Science 126:400, 1957.

Axelrod, J., Inscoe, J.K., Senoh, S. and Witkop, B.: O-Methylation, the principle pathway for the metabolism of epinephrine and norepinephrine in the rat. Biochim. biophys. Acta 27:210, 1958a.

Axelrod, J., Senoh, S. and Witkop, B.: O-Methylation of catecholamines in vivo. J. biol. Chem. 233:697, 1958b.

Axelrod, J. and Tomchick, R.: Enzymatic O-methylation of catecholamines. J.biol. Chem. 233:702, 1958.

Axelrod, J.: The metabolism of catecholamines in vivo and in vitro. Symposium on Catecholamines, Pharmacol. Rev. 11:402, 1959.

Bertler, A., Carlsson, A. and Rosengren, E.: A method for the fluorimetric determination of adrenaline and noradrenaline in tissues. Acta physiol. scand. 44:273, 1958.

Bessinger, H. and Wakerlin, G.: Relation of adrenal glands to renin concentration of the canine kidney. Amer. J. Physiol. 155:426, 1948.

Best, C.H. and Taylor, N.B.: The Physiological Basis of Medical Practice. 6th Edit. Williams and Wilkins Co., Baltimore, 1956.

Bing, R.J.: The formation of hydroxytyramine by extracts of renal cortex and by perfused kidneys. Amer. J. Physiol. 132:497, 1941.

Blaschko, H.: The specific action of l-dopa decarboxylase. J. Physiol. 96:50P, 1939.

Blaschko, H., Holton, P. and Stanley, G.: Enzymatic formation of pressor amines. J. Physiol. 108:427, 1949.

Blaschko, H.: Amine oxidase and amine metabolism. Pharmacol. Rev. 4: 415, 1952.

Bowman, R.L. and Udenfriend, S.: Spectrophotometric assay throughout the ultra-violet and visible range. Science 122:32, 1955.

Brandt, W., Dubin, M. and Sapoirstein, L.: Studies on salt hypertension effects on adrenalectomy and nephrectomy. Amer. J. Physiol. 164:75, 1951.

Burn, J.H. and Rand, M.J.: Reserpine and noradrenaline in artery walls. Lancet 2:1097, 1957.

Burn, J.H. and Rand, M.J.: The depressor action of dopamine and adrenaline. Brit. J. Pharmacol. 13:471, 1958.

Carlsson, A. and Waldeck, B.: A fluorimetric method for the determination of dopamine (3-hydroxytyramine). Acta physiol. scand. 44:293, 1958.

Collins, D.A.: Hypertension from constricted arteries of denervated kidneys. Amer. J. Physiol. 116:616, 1936.

Cotzias, G.G. and Dole, V.P.: The microdetermination of monamine oxidase in tissues. J. biol. Chem. 90:665, 1951.

Crawford, T.B.B. and Law, W.: A method for the estimation of adrenaline and noradrenaline in urine. J. Pharm. and Pharmacol. 10:179, 1958.

Crout, R., Creveling, C.R. and Caton, D.: Metabolism of norepinephrine by rat brain and heart. Fed. Proc. 19:297, 1960.

Demis, D.J., Blaschko, H. and Welch, A.D.: The conversion of dihydroxyphenylalanine to -2C^{14} (DOPA) to norepinephrine by bovine adrenal homogenates. J. Pharmacol. exp. Therap. 113:14, 1955.

Demis, D.J., Blaschko, H. and Welch, A.D.: The conversion of dihydroxyphenylalanine to -2C^{14} (DOPA) to norepinephrine by bovine adrenal homogenates. J. Pharmacol. exp. Therap. 117:200, 1956.

Dock, W.: Vasoconstriction in renal hypertension. Amer. J. Physiol. 1:1, 1940.

Drujan, B.D., Sourkes, T.L., Layne, D.S. and Murphy, G.F.: The differential determination of catecholamines in urine. Canad. J. Biochem. Physiol. 37:1153, 1959.

Duggan, D.E., Bowman, R.L., Brodie, B.B. and Udenfriend, S.: A spectrophotofluorometric study of compounds of biological interest. Arch. Biochem. 68:1, 1957.

Dury, D.R.: The production of hypertension in the rabbit by a new method of renal insufficiency. J. exp. Med. 68:695, 1938.

Euler, U.S. von and Hellner, S.: Excretion of adrenaline, noradrenaline and hydroxytyramine in the urine. Acta physiol. scand. 22:161, 1951.

Euler, U.S. von: Noradrenaline. Charles C. Thomas, Springfield, Ill., 1956.

Gaddum, J.H.: Bioassay procedures. Symposium on catecholamines. Pharmacol. Rev. 11:241, 1959.

Gey, K.F. and Pletscher, A.: Influence of chlorpromazine and chlorprothixene on the cerebral metabolism of 5-hydroxytryptamine, norepinephrine and dopamine. J. Pharmacol. exp. Therap. 133:18, 1961.

Giordano, C., Samily, A.H., Bloom, J., Haynes, F.W. and Merrill, J.E.: Studies on experimental hypertension in rabbits. Fed. Proc. 100:19, 1959

Goldblatt, H., Lynch, J., Hanzel, R.F. and Summerville, W.W.: Studies on experimental hypertension. J. exp. Med. 59:347, 1934.

Goldblatt, H.: Factors regulating blood pressure. J. Macy Found. 8th Conference, 1951.

Goldring, W.: A note on the history of hypertension. Experimental Hypertension. Spec. Pub. of the N.Y. Acd. Sciences. Vol. 3, 1946.

Goldstein, M., Freidhoff, A. and Simmons, C.: Metabolic pathways of 3-hydroxytyramine. Biochim. biophys. Acta 33:377, 1959.

Goodall, McC.: Dihydroxyphenylalanine and hydroxytyramine in mammalian suprarenals. Pharmacol. Rev. 4:350, 1950.

Goodal, McC.: Studies on adrenaline and noradrenaline in mammalian heart and suprarenals. Acta physiol. scand. 24: suppl. 85, 1951.

Goodall, McC. and Kirshner, N.: Biosynthesis of adrenaline and noradrenaline in vitro. J. biol. Chem. 226:213, 1957.

Goodall, McC.: Metabolic products of adrenaline and noradrenaline in human urine. Symposium on Catecholamines. Pharmacol. Rev. 11:416, 1959.

Gregerman, R.I.: Hydroxytyramine in the parotid gland venom of the toad, Bufo-marinus. J. gen. Physiol. 35:485, 1951.

Grollman, A.C., Harrison, R. and Williams, J.: The mechanism of experimental renal hypertension in the rat. J. exp. Med. 59:374, 1934.

Grollman, A.C. and Rule, G.: Experimentally induced hypertension in parabiotic rats. Amer. J. Physiol. 113:587, 1938.

Grollman, A.C.: Experimental chronic hypertension in the rabbit. Amer. J. Physiol. 142:666, 1942.

Grollman, A.C.: A simplified procedure for inducing chronic renal hypertension in the rabbit. Proc. Soc. exp. Biol., N.Y. 57:102, 1944.

Hagen, P. and Welch, A.: The adrenal medulla and the biosynthesis of pressor amines. Recent Progr. Hormone Res. 12:27, 1956.

Halle, E.K. and Hall, O.: Persistence of DCA-induced hypertension in the nephrectomised rats. Proc. Soc. exp. Biol., N.Y. 70:690, 1949.

Haynes, F., Forsham, P. and Thorn, D.: Effects of ACTH, cortisone, DCA, and epinephrine on the plasma hypertensinogen and renin concentrations of dogs. Amer. J. Physiol. 172:268, 1953.

Holtz, P., Heise, R. and Ludtke, K.: Fermentativer abbau von l-dioxyphenylalanin (Dopa) durch niere. Arch. exp. Path. Pharmak. 191: 87, 1938.

Holtz, P.: Dopa decarboxylase. Naturwissenschaften 27:724, 1939.

Hornykiewicz,O.: The action of dopamine on the arterial blood pressure of the guinea pig. Brit. J. Pharmacol. 13:191, 1958.

Horowitz, D., Goldberg, L.I., Sjoerdsma, A. and Ambrose, I.: Increased blood pressure responses to dopamine and norepinephrine produced by monoamine oxidase inhibitors in man. J. Lab. clin. Med. 56:747, 1960.

Houdobro, F. and Braun-Menendez: Secretion of renen by the intact kidney. Amer. J. Physiol. 137:47, 1942.

Houssay, B.A. and Taquini, A.C.: Rev. Soc. Biol. 14:86, 1938. (Cited by Coke, Experimental Hypertension, 1946).

James, W.O.: Demonstration and separation of adrenaline, noradrenaline, and methyl adrenaline. Nature 161:651, 1948.

Kirshner, N. and Goodall, McC.: Biosynthesis of adrenaline and noradrenaline by adrenal slices. Fed. Proc. 15:110, 1956.

Langemann, H.: Enzymes and substrates in the adrenal gland of the Ox. Brit. J. Pharmacol. 6:318, 1951.

Leeper, L.C. and Udenfriend, S.: 3,4-Dihydroxyphenylethylamine as a precursor of adrenal epinephrine in the intact rat. Fed. Proc. 15: 298, 1956.

Manger, W.M., Wakim, K.G. and Bollman, J.L.: Chemical Quantitation of Epinephrine and Norepinephrine in Plasma. Charles C. Thomas, Springfield, Ill., 1959.

Mason, H.C., Corcoran, A.C. and Page, I.H.: The comparative activities of 17-hydroxy-11-desoxycortico-sterone. Endocrinology 46:441, 1950.

Menendez, Braun: The pharmacology of renin and hypertensin. Pharmacol. Rev. 8:25, 1956.

Ostle, B.: Statistics in Research, The Iowa State College Press, Ames Iowa, 1954.

Page, I.H.: Pressor substances from the fluids of man in health and disease. J. exp. Med. 61:67, 1935.

Page, I.H. and Sweet, J.E.: Effect of hypophysectomy on the arterial blood pressure of dogs with experimental hypertension. Amer. J. Physiol. 120:238, 1937.

Page, I.H.: The production of persistent arterial hypertension by cellophane perinephritis. J. Amer. med. Ass. 113:2046, 1939a.

Page, I.H.:A method for the production of persistent hypertension by cellophane. Science 89:273, 1939b.

Page, I.H.: Demonstration of liberation of renin into the blood stream from kidneys of animals made hypertensive by cellophane perinephritis. Amer. J. Physiol. 130:22, 1940.

Page, I.H., Helmer, O.M., Kohlstaedt, K.G., Fouts, P.J. and Kempf, G.H.: The reduction of arterial blood pressure in hypertensive patients and animals with extracts of kidneys. J. exp. Med. 73:7, 1941.

Progund, S.S., Drell, W. and Clark, W.G.: The metabolism of 3-hydroxy- and 3,4-dihydroxyphenylpyruvic acids in vivo. J. Pharmacol. exp. Therap. 131:294, 1961.

Saplerstein, L., Ogden, E. and Southard, F.: Renin-like substances in the blood after hemmorhage. Proc. Soc. exp. Biol. Med. 48:503, 1941.

Sevy, R.W. and Wakerlin, G.E.: Endocrine factors in experimental hypertension. Amer. J. Physiol. 172:129, 1953.

Sheline, R.R., Williams, R.T. and Wit, J.G.: Biological dehydroxylation. Nature 188:849, 1960.

Shepherd, D.M. and West, G.B.: Hydroxytyramine and the adrenal medulla. J. Physiol. 120:15, 1953a.

Shepherd, D.M. and West, G.B.: Detection of some precursors of adrenaline by paper chromatography. Nature 171:1160, 1953b.

Shipley, R.E., Helmer, O.M. and Kohlstaedt, K.G.: The presence in blood of a principle which elicits a sustained pressor response in nephrectomized animals. Amer. J. Physiol. 149:708, 1948.

Shore, P. and Olin, T.: Identification and chemical assay of norepinephrine in brain and other tissues. J. Pharmacol. exp. Therap. 122:295, 1958.

Schroeder, H. and Adams, E.: The effect of tyrosine on experimental hypertension. J. exp. Med. 73:531, 1941.

Schroeder, H.: The effect of a preparation of amine oxidase on experimental hypertension. Science 93:305, 1942.

Sjoerdsma, A., Lovenberg, W., Oates, J.A., Crout, J.R. and Udenfriend, S.: Alterations in the pattern of amine excretion in man produced by a monoamine oxidase inhibitor. Science 130:225, 1959.

Spector, S., Kuntzman, R., Shore, P.A. and Brodie, B.B.: Biochemical and pharmacological effects of the monoamine oxidase inhibitors, iproniazid, 1-phenyl-2-hydrazinopropane (JB 516) and 1-phenyl-3-hydrazinobutane (JB 835). J. Pharmacol. exp. Therap. 128:15, 1960a.

Spector, S., Kuntzman, R., Shore, P.A. and Brodie, B.B.: Evidence for the release of brain amines by reserpine in the presence of monoamine oxidase inhibitors. Implication of monoamine oxidase in norepinephrine metabolism in the brain. J. Pharmacol. exp. Therap. 130:256, 1960b.

Tiegerstedt, R. and Bergman, R.: Skand. Arch. Physiol. 8:223, 1898. (Cited by Best and Taylor, 1956).

Udenfriend, S.: Survey of chemical and physical methods for measuring catecholamines. Symposium on Catecholamines. Pharmacol. Rev. 11:252, 1959

Weil-Malherbe, H. and Bone, A.D.: The estimation of catecholamines in the urine by a chemical method. J. clin. Path. 10:138, 1957.

VITA

David Rockwell De Fanti was born November 12, 1932, in Wakefield, Rhode Island, the son of Mr. and Mrs. John De Fanti, Jr.

He was graduated from Colgate University, Hamilton, New York, in 1955 with the degree of Bachelor of Arts in Zoology. He enrolled in the University of Rhode Island, College of Arts and Sciences, in 1955 and was graduated in 1957 with the degree of Master of Science in Zoology. He continued his graduate education in the College of Pharmacy, Department of Pharmacology, at the University of Rhode Island and completed the requirements for the degree of Doctor of Philosophy in November, 1961. From July, 1959 through July, 1961 he held a United States Public Health Service Research Fellowship and in September, 1961 was appointed Assistant Professor of Pharmacology at the University of Rhode Island.

He is a member of the following honor societies: Rho Chi, Phi Sigma and Sigma Xi.

He married Mary Catherine Bourret in 1957 and has one child, a son, John.